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PRINCIPAL INVESTIGATOR: Jayant P. Agarwal, MD

RECIPIENT: University of Utah
Salt Lake City, UT 84112

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Jayant P. Agarwal and Jill E. Shea
E-Mail: Jay.Agarwal@hsc.utah.edu and Jill.Shea@hsc.utah.edu

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University of Utah
30 North 1900 East #3B400,
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14. ABSTRACT

Nerve injury can occur due to penetrating wounds, compression, traumatic stretch, and cold exposure. Despite prompt repair, outcomes are dismal. In this work, a poly(lactic-co-glycolic acid)(PLGA) nerve conduit with associated biodegradable drug reservoir is designed, fabricated, tested. Devices loaded with nerve growth factor (NGF) are evaluated for sustained drug release and axon growth enhancement in dorsal root ganglion (DRG) cells with the released drug. In the first year of this 18 month project we have completed device fabrication of the nerve guide conduit and drug delivery reservoir. We were able to release NGF at a concentration that enhancing DRG nerve growth *in vitro*. We next compared functional recovery between an autograft, PLGA nerve conduit, and PLGA nerve conduit that releases NGF in a rat sciatic nerve gap model. The animals that had the nerve gap repaired with an autograft had less muscle atrophy and greater neuromuscular junction connectivity compared with animals that received either the conduit alone or the conduit that released NGF. There were no functional differences between the two PLGA conduit groups.

15. SUBJECT TERMS Peripheral nerve regeneration, nerve conduit, nerve growth factor, poly lactic co-glycolic acid, drug-delivering conduit, axon elongation, drug delivery device

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

Combat gear for the modern day warrior has greatly improved protection for the head and body, but limbs are still highly exposed to injury. Subsequently, the most frequent combat nerve injuries are in the upper and lower extremities. In terms of the general population and non-combat veterans peripheral nerve injuries affect 2-3% of trauma patients and vastly more subsequent to tumor extirpation or iatrogenic injury. Patients often suffer from life-long loss or functional disturbances mediated by the injured nerve, which can severely diminish their quality of life. Unfortunately, current treatments often result in inadequate or untimely repair, which can result in lifelong deficits in muscle function or sensation. Nerve injuries with large gaps (>1cm) require special bridging strategies for tension-free repair. Autologous nerve grafts serve as the state-of-the-art in repairing such gaps but numerous challenges associated with this approach results in functional benefits to only 40-50% patients. Much progress has been made in the field of artificial nerve conduits with collagen and poly(lactic-co-glycolic acid)(PLGA) conduits commercially available and in use. These hollow tubes act as axon guides for the regenerating nerves and can allow for tension free bridging without the need to harvest donor nerve. A number of research groups have proposed conjugating drugs into these conduits or using other biodegradable components such as hydrogels. The shortcomings of current devices in terms of burst effect, nonuniform dosage, and uneven drug delivery, necessitates a new approach to deliver drug for nerve regeneration. This project focuses on a novel approach to deliver drugs to a regenerating nerve in a controlled manner. This unique design consists of a biodegradable drug delivery device, capable of delivering proteins and small molecules at zero order kinetics, attached to a biodegradable PLGA conduit. The drug delivery device consists of three main components: (i) a drug reservoir, (ii) a biodegradable polymer matrix for controlled drug delivery, and (iii) a diffusion hole for controlled drug release. We will study the efficacy of our novel biodegradable nerve conduits to (1) continuously deliver small molecules or growth factors to the regenerating axons at a controlled rate and (2) improve the degree of axon regeneration and functional recovery. This project will focus on the local delivery of nerve growth factor (NGF), a protein, which has been shown to enhance peripheral nerve regeneration.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Peripheral nerve regeneration, nerve conduit, nerve growth factor, poly lactic co-glycolic acid, drug-delivering conduit, axon elongation, drug delivery device

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.
***Specific Aim 1* -- To optimize release kinetics of NGF *in vitro* using our novel drug delivery conduit**

1. Manufacture Devices for use in 15mm nerve gap .....(Gale) (months 0-5)
    a. Optimize PLGA ratios.....(Gale)(months 0-1)
    b. Optimize nanoporous membrane dimensions.....(Gale)(months 2-3)
    c. Optimize reservoir dimensions .....(Gale)(months 3-4)
    d. Manufacture and assemble components .....(Gale)(months 4-5)

2. In Vitro NGF release kinetics experiments.....(Gale,Agarwal) (months 5-8)

3. HPLC/ELISA detection of NGF .....(Ambati)
(months 5-8)

***Specific Aim 2* -- To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a 15mm nerve gap in a rat sciatic nerve model.**
Tasks/Subtasks:

1. IACUC approval, obtain N=55 animals (48 experimental animals) (We are requesting 7 additional animals from IACUC for possible early losses) .....(Agarwal)
(months 8-10)

2. Experimental Groups 1-3 (n=16/group) .....(Agarwal)
(months 10-14)
    a. Sacrifice of half animals at day 21 (n=8/group) .....(Agarwal)
    (months 10-12.5)
    b. HPLC/ELISA for NGF detection of day 21 animals (n=8/group)
    .....(Ambati)
    (months 12.5-13.5)
    c. Sacrifice of half animals at day 90 (n=8/group) .....(Agarwal)
    (months 13-14)
    d. Walking Track(all animals n=16/group).....(Agarwal)
    (months 10-14)

3. Explanted tissue analysis .....(Agarwal,Ambati)
(14-16 months)
    a. HPLC/ELISA for NGF detection of day 90 animals (n=8/group)
    .....(Ambati)
    (months 14-15.5)
    b. Nerve histology and IHC .....(Agarwal)
    (months 14-16)
    c. Muscle Histology .....(Agarwal)
    (months 14-16)

4. Data analysis and Manuscript Preparation
(Agarwal,Gale,Ambati)(months 17-18)

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) MAJOR ACTIVITES
***Specific Aim 1* -- To optimize release kinetics of NGF *in vitro* using our novel drug delivery conduit**

Tasks/Subtasks:
1. Manufacture Devices for use in 15mm nerve gap **completed**
    a. Optimize PLGA ratios ..... **completed**
    b. Optimize diffusion hole dimensions ..... **completed**

c. Optimize reservoir dimensions……..…… ..... **completed**
d. Manufacture and assemble components……. ..... **completed**

2. In Vitro NGF release kinetics experiments..... **completed**

3. HPLC/ELISA detection of NGF……..... **completed**

Progress: We have completed the three main tasks of Aim 1.

***Specific Aim 2* -- To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a 15mm nerve gap in a rat sciatic nerve model.**

Tasks/Subtasks:
1. IACUC/ACURO approval (n=48 animals)……..... **completed**

2. Experimental Groups 1-3 (n=16/group)……..... **completed**
   a. Sacrifice of half animals at day 21 (n=8/group)
   completed
   b. HPLC/ELISA for NGF detection of day 21 animals (n=8/group)
   completed
   c. Sacrifice of half animals at day 90 (n=8/group)
   completed
   d. Walking Track (90 Day groups; n=8/group)
   completed

3. Explanted tissue analysis ..... **in progress**
   a. HPLC/ELISA for NGF detection of day 90 animals (n=8/group)
   completed
   b. Nerve histology and IHC
   in progress; 75% complete
   c. Muscle Histology
   in progress; 75% complete

4. Data analysis and Manuscript Preparation..... **in progress**; 50% complete

Progress: We have completed 90% of the tasks in Aim 2. We have finished the surgeries, walking track, analysis of NGF in device/surrounding tissue, and muscle atrophy. We are finishing analyzing and compiling the nerve histology and retrograde labeling.

2) SPECIFIC OBJECTIVES
1. To optimize release kinetics of NGF *in vitro* using our novel drug delivery conduit
2. To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a 15mm nerve gap in a rat sciatic nerve model.

3) SIGNIFICANT RESULTS
***Specific Aim 1* -- To optimize release kinetics of NGF *in vitro* using our novel drug delivery conduit**
Material Fabrication
The proposed device consists of two concentric tubes, a reservoir in between the tubes that stores the drug, and a hole(s) within the inner tube that enables the drug to be released into the lumen (**Figure 1**). The bioresorbable guidance conduits were manufactured using 75/25 poly-lactic-glycolic-acid (PLGA; 7525 DLG 7E, Evonik). The individual PLGA device components are shown in **Figure 2**. The solvent casting method was used for manufacturing devices, with the 75/25 PLGA pellets dissolved in acetone at a ratio of 2:3 at 45°C. Ethanol was

then added to the solution, stirred until visibly homogenous, and then the solution was used to fill glass molds. Glass tubing of various sizes were utilized as molds to construct the PLGA inner and out condiut (**Figure 1 and Figure 2**). The end caps were made using a flat petri dish mold, which resulted in 1.5mm thick sheets.

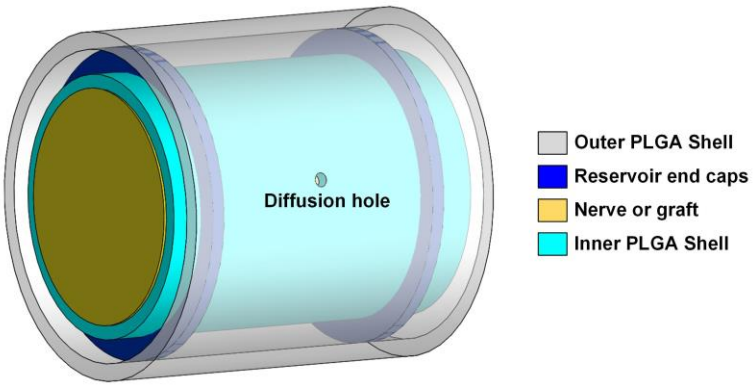


Figure 1. *Schematic of nerve drug delivery device demonstrating key structural characteristics including: Inner (light blue) and outer (grey) PLGA shell, drug reservoir (space between shells), and end caps defining boundary of reservoir (dark blue.) The diffusion hole (in the inner shell) can be modified to control the rate of diffusion.*

Immediately following the suctioning of material into the conduits, any excess PLGA was allowed to drip out of the molds. The conduits were then placed vertically into a water bath and allowed to cure. The water bath quickly displaced the solvent and reduced deformations caused by gravity on the conduit. Conduit dimensions were determined based on studies for sciatic nerve repair for rats. The rat inner conduits were molded to dimensions of 2.4mm OD and 2mm ID and also cut to a length of 13mm. The outer conduits measured 4mm OD and 3mm ID and were cut to a length of 7mm.

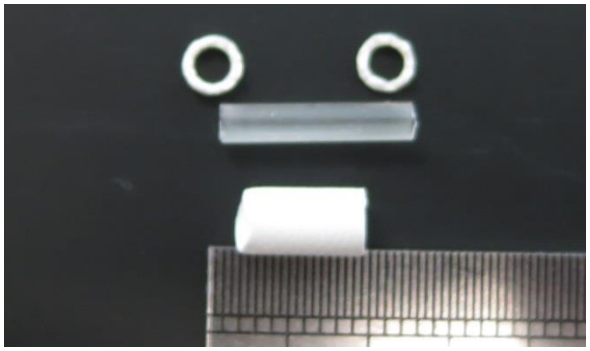


Figure 2. Conduit Assembly - inner conduit, outer conduit, and end caps

End caps to join the conduits and form the drug reservoir were manufactured by forming a PLGA sheet. The same PLGA solution that was used for the conduits was poured on to a glass petri dish and left on a 45°C hot plate. This heating process cures the sheet in a bottom-up process that minimizes wrinkling of the sheet as it expands and contracts. Following 4 hours on a hot plate, the sheet was submerged into water, allowing the emulsion process to remove any remaining solvent. The end caps were cut to size using a laser cutter. A properly sized end cap creates a slight press fit over the inner conduit and within the outer conduit.

<u>Conduit Assembly</u>

Following fabrication of the inner conduit, the diffusion holes are drilled into the devices using laser milling. We are able to drill holes with diameters between 15µm and 100µm using the MicroMaster KrF excimer laser system (Optec) and with diameters larger than 100µm using the VLS3.60 CO_2 system (Universal Laser Systems)) (**Figure 3**). The laser holes were stabilized by pulsing the laser 40 times. This process annealed the PLGA surrounding the diffusion holes and made them more stable once submersed into water.

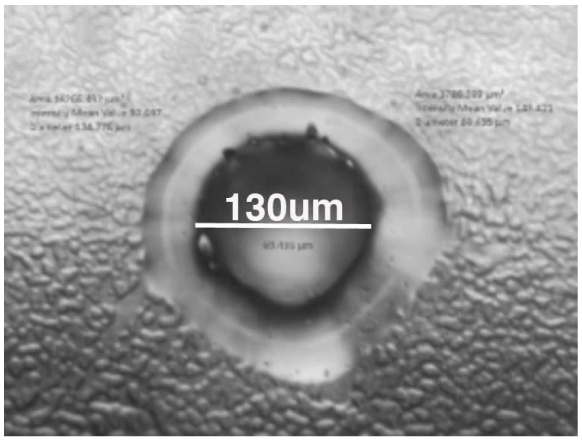


Figure 3. *Image of an inner condiut with a 130µm laser drilled hole.*

A solvent bonding process was adopted to assemble and seal the inner and outer PLGA conduits. A solution consisting of PLGA and acetone was mixed together to form a viscous solution. The solution was locally applied to join the end caps to the inner conduit (**Figure 4**). Following each application of solvent glue, the devices were placed into a water bath for 24 hours to remove any residual solvents and return the assembly to a solid structural state.

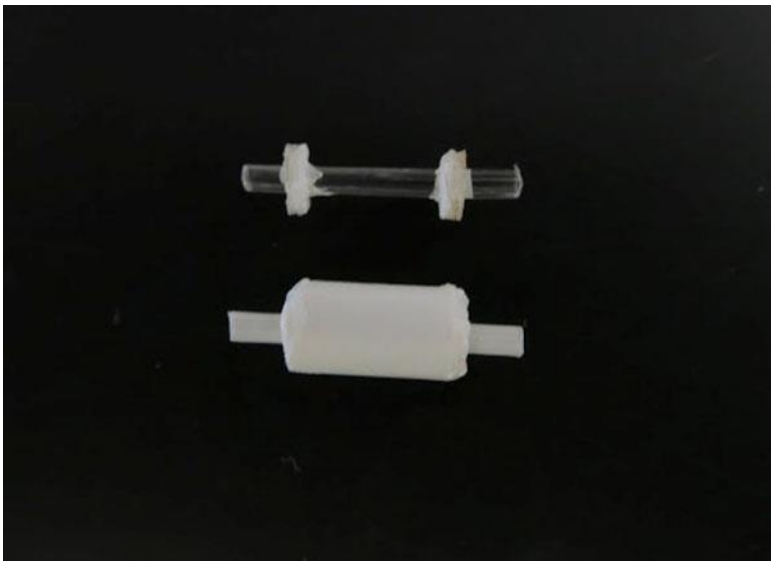


Figure 4*. (Top) Inner conduit with two end caps defining the outer boundary of the reservoir. (Bottom) Completed PLGA device with inner and outer tube, as well as a diffusion hole on the inner conduit that enables the release of NGF from the reservoir into the inner chamber.*

The final assembly step involved solvent welding the outer conduits to complete the drug reservoir (Figure 4; bottom image). Once the outer conduit was attached, the devices were again placed in a water bath to displace any remaining acetone

<u>Sterilization</u>

PLGA poses a few problems regarding sterilization. It is a reactive polymer that may break down from certain radiation or chemical sterilization processes. Additionally, the fragile co-polymer has very low melting point and even lower glass transition temperature (40-60°C), eliminating many traditional sterilization methods like autoclaving. We evaluated deformation of the device and diffusion hole follow sterilization. The manufactured PLGA devices were sterilized using 70% ethanol (n=42), ethylene oxide (ETO) (n=46), and a Sterrad 100S plasma sterilization (n=50) process. The results indicated minimal hole deformation from the ethanol and Sterrad sterilization processes but a complete collapse of holes in ETO-sterilized samples. As a

result, the Sterrad 100S system was chosen as the sterilization method for our PLGA conduits due to minimal degradation and deformation and verified sterility.

Release Kinetics Fluorescently Labeled Dextran

While guidance conduits for peripheral nerve regeneration have been a key area of study for the past 30 years, they have not yet supplanted the autograft. By relying on diffusion as the primary delivery mechanism, our device avoids complex and sometimes unreliable degradation rates of the polymers that encase the target drug. Since the drug delivery is based on diffusion, structural characteristics of the device such as diffusion hole size and reservoir size, can be used to predict the diffusion characteristics of a drug prior to actually building the device. Therefore in the process of developing our device, we have created a mathematical model based on Fick's law that allows us to estimate how changing the volume of the reservoir, concentration of drug, and hole size alter release kinetics (**Figure 5**; orange line). The one dimensional diffusion model was created within MATLAB enabling us to predict diffusion rates based on various input parameters such as diffusion coefficient, diffusion area, diffusion distance, and reservoir volume. We utilized this analytical model to assist in the development of our initial drug delivery device. We were able to accurately predict the release of fluorescently labeled dextran, with a similar diffusion coefficient to NGF, over a period of approximately 40 days (**Figure 5**; blue line). The sealed device, with no diffusion hole, released very little dextran suggesting the drug is primarily released through the diffusion hole and does not permeate through the walls (**Figure 5**; green line). Additionally, since our device doesn't rely on polymer degradation for drug release, we are able to easily change delivery parameters for different drugs and minimize initial burst release.

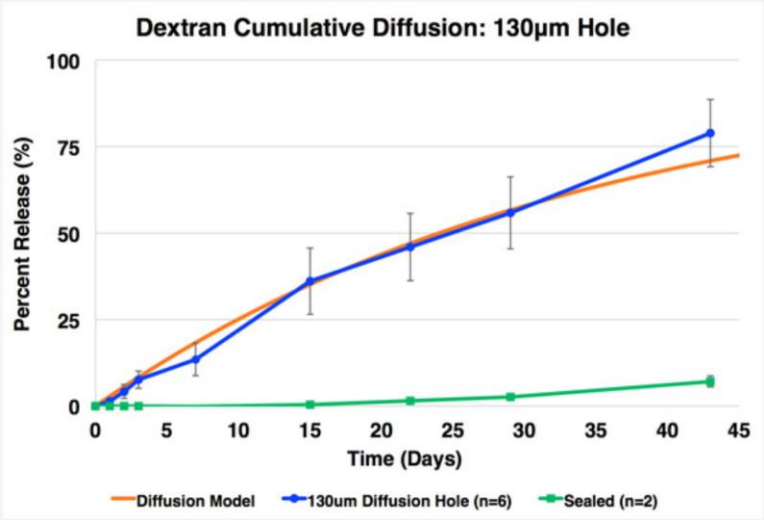


Figure 5. *Fluorescently labeled dextran (blue) was released from our drug reservoir device (n=4) for approximately 40 days, with data comparing well to our model predication (orange).*

Release Kinetics NGF

We next evaluated the release of NGF from the device at two different concentrations with a 130µm diffusion hole. We have previously evaluated the growth of DRGs exposed to NGF and determined that dosages ranging from 0.1ng/mL to 100ng/mL all enhanced axonal extension. Specifically, DRGs exposed to NGF concentrations of 0.1ng/mL (910.5 ± 39.8µm), 1ng/mL (993.7 ± 53.6 µm), 5ng/mL (1063.5 ± 57.8 µm), 10ng/mL (1117.6 ± 55.6 µm) and 100ng/ml (755.7 ± 57.8 µm) all had statistically longer axons when compared to controls 0ng/mL (469.7 ± 23.4; $p<0.05$) (**Figure 6**). Our goal was to release NGF from the device at a concentration between 0.1-50ng/mL. We observed that we were able to release NGF at a consistent dosage that was below 50ng/mL and above 1ng/mL, with larger concentrations observed when 0.5mg/mL were loaded into the reservior compared with 0.25mg/mL (**Figure 7**).

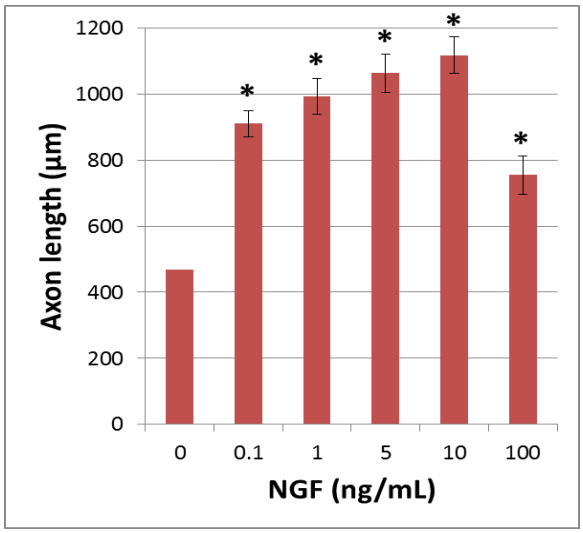


Figure 6. *NGF enhances axon length at dosages ranging from 0.1-100ng/mL. *statistically different from control 0<0.05)*

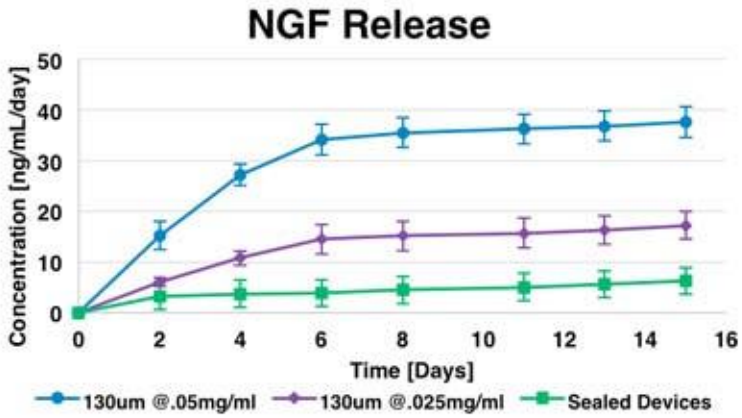


Figure 7. *NGF was released from our PLGA nerve conduit device loaded with two different NGF concentrations. The released NGF was within a range that has been shown to enhance axon growth.*

In summary we successfully manufactured a nerve conduit device that consists of an inner conduit, outer conduit, and drug reservoir (**Figure 1**). The device is composed of entirely of PLGA and can release NGF at a concentration that to enhances nerve growth *in vitro*. Therefore, we were able to achieve the major objectives of Aim 1 and then progressed towards Aim 2.

Specific Aim 2 **-- To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a 15mm nerve gap in a rat sciatic nerve model.**

The efficacy portion of this grant was designed to compare functional recovery using a rat sciatic nerve injury model. We compared functional recovery between animals that had a gap injury repaired with an autograft, conduit, or conduit that released NGF. The main outcome measures were muscle atrophy and sciatic functional index.

<u>Conduit</u>

At the 21 day harvest we observed that the PLGA conduits in both the conduit alone and conduit with NGF did not maintain their structure (**Figure 8**). It appeared that the inner conduit and outer conduits were compressed resulting in a significantly smaller inner conduit diameter. Also, it appeared the reservoir was collapsed. We measured the amount of NGF in the reservoir and serum at 21 days and observed that there was no detectable NGF within the reservoir and no difference in the serum levels of NGF between the autograft (7.8±7.5ng/mL), conduit (0.5±0.2ng/mL), and conduit + NGF (3.2±2.9ng/mL). At 180 days the conduit in both the conduit alone and the conduit with NGF had completely degraded. However, the sutures were still present at the proximal and distal ends of the nerve gap. One interesting observation was that the distance between the sutures was not consistent. At the time of implantation all groups had a nerve gap length of 10mm. At harvest the distance between sutures in the autograft group had a range of 1.0-1.4cm, the conduit alone a range of 0.9-

1.9 cm, and the conduit with NGF a range of 1.0-2.5 cm. The variability in gap length between sutures is likely the result of the conduit degrading prior to nerve regeneration and the sutures pulling apart. The animals with sutures that were farther apart had a much greater distance that the nerve had to regenerate compared to animals that had a shorter gap length.

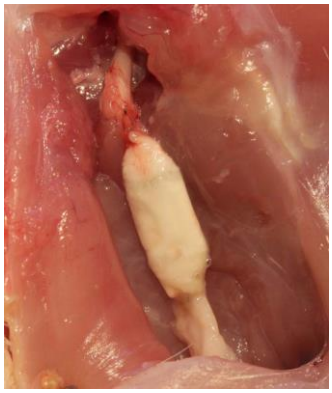


Figure 8. The PLGA nerve conduit device after 21 days implantation in a rat sciatic nerve gap model. The device compressed resulting in a smaller inner conduit diameter. It is also possible that leaks could have formed within the reservoir as a result of the deformation.

Walking Track

We evaluated the gait of the rats prior to surgery and every other week post surgery for the length of the experiment (24 weeks)(**Figure 9**). All animals exhibited a dramatic decrease in terms of the sciatic functional index (SFI) at two weeks post surgery. All groups had small increases in measures of SFI with time, but never reached pre surgery levels. There were no group differences.

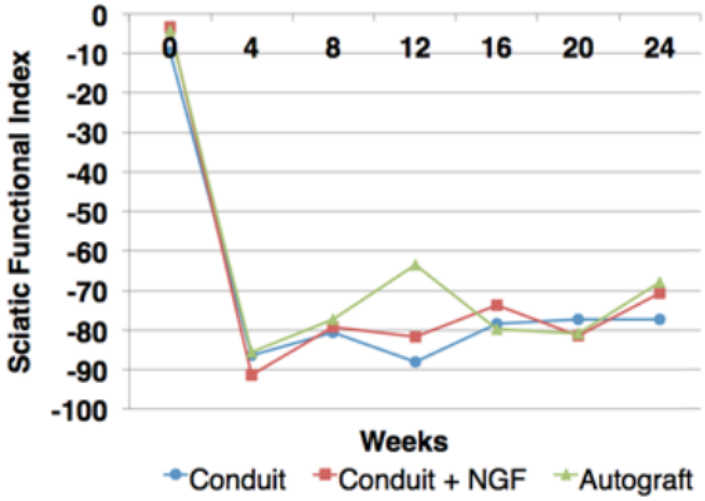


Figure 9. *Graph of the sciatic functional index (SFI) over the coarse of the experiment. All groups had a decrease in SFI at two weeks post surgery with only incremental increases with time. There were no group differences prior to surgery at week 2 or week 24.*

Muscle Atrophy

The gastrocnemius of both legs were harvested at necropsy, with care taken to dissect at the tendinous origin and insertion. The muscles were then weighed and the degree of relative atrophy between the experimental and control muscle calculated [(experimental-control)/control *100)]. At 21 days there were no group differences between the autograft, conduit, and conduit with NGF in terms of gastrocnemius muscle atrophy (**Figure 10**). The gastrocnemius of the treated side was approximately 60% of the control side for all experimental groups. This is in contrast with the muscle atrophy results at 180 days where the autograft group had statistically less muscle atrophy than the conduit group or the conduit with NGF group (**Figure 11 and 12**). Although the autograft side still exhibited signs of muscle atrophy the muscle had recovered more than the two groups that

had the nerve gap repaired with the conduit. Since there was variability in the gap length at the 180 day harvest (see conduit section above) we also evaluated muscle atrophy normalized to gap length (distance between the proximal and distal sutures). The autograft still had less muscle atrophy but there was a trend for the conduit with NGF having less atrophy than the conduit alone (p=0.13).

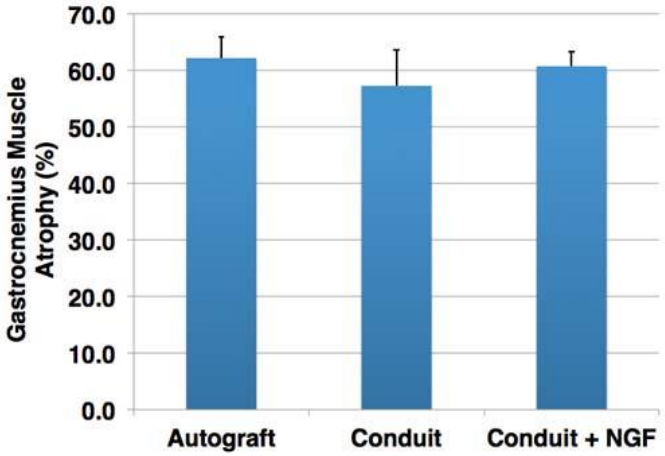


Figure 10. *There were no group differences in terms of gastrocnemius muscle atrophy at 21 days between the three experimental groups.*

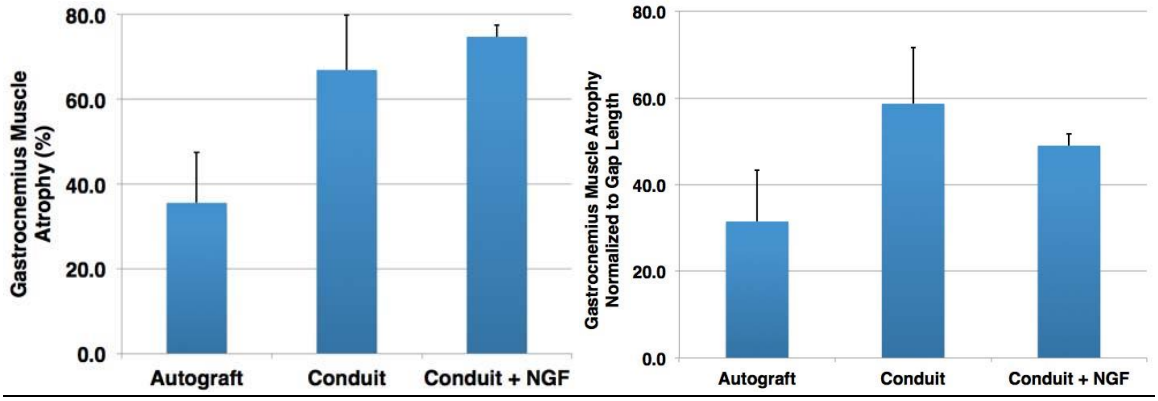


Figure 11. (Left) The PLGA device with NGF (NGF) and the PLGA device with no drug (ND) had significantly greater muscle atrophy 6 months post implantation compared to the autograft group ($p<0.05$). (Right) As a result of degradation of the PLGA the nerve gap was not consistent between animals at the time of harvest. Thus atrophy was normalized to the distance between the sutures (proximal and distal) at harvest.

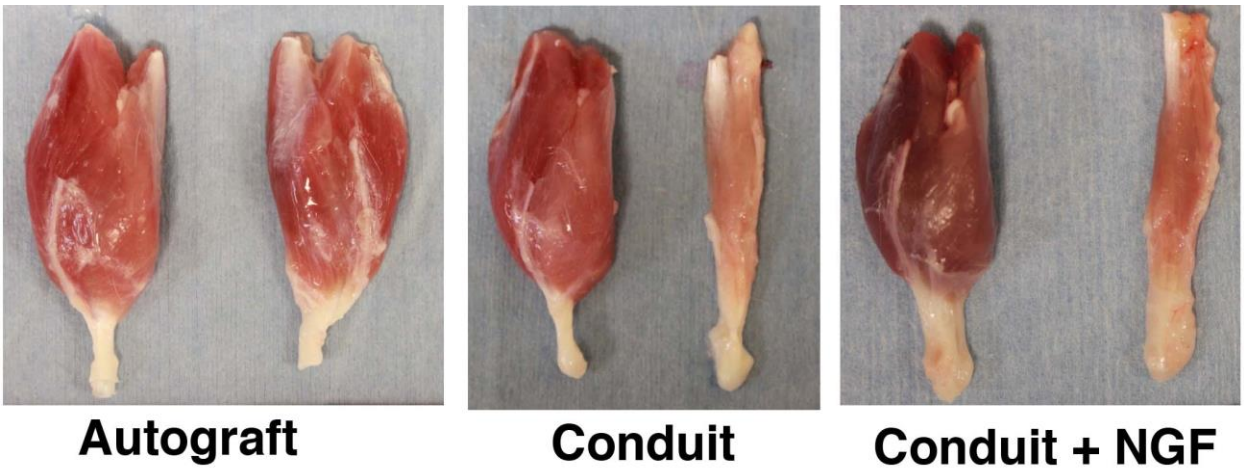


Figure 12. *Photographs of the control gastrocnemius muscle (left) and the treated muscle (right) for the three experimental groups. The autograft group had less muscle atrophy compared with the two conduit groups.*

Neuromuscular Junction

At 180 days we evaluated the degree of connectivity between acetylcholine receptors and nerve fibers. At harvest the animals were perfused first with heparinized saline and then with 2% paraformaldehyde solution. Following whole body perfusion the soleus muscle will be harvested, fixed in 2% paraformaldehyde for 2 hours, cyroprotected in sucrose, embedded in optimum-cutting-temperature compound, and then frozen sectioned (≈25μM). The frozen sections will be used to analyze neuromuscular junction connectivity and density. The sections will be stained with alpha-bungarotoxin conjugated to Alexa 594 (1:500; Molecular Probes, OR) to label the acetylcholine receptors and neurofilament (1:25; Biolegend, CA) and synaptic vessel protein (SV2)(1:25 Developmental Studies Hybridoma Bank, IA) to label the nerve fibers. The data is reported as the percentage of acetylcholine receptors connected to nerve fibers. There were no differences in the number of acetylcholine receptors connected to nerve fibers between the untreated control side and the autograft group (**Figure 13**)($p=0.22$) and between the conduit and conduit with NGF groups ((**Figure 13**)($p=0.99$). Both the control side and autograft group had more receptors attached to nerve fibers compared with the conduit and conduit and NGF ($p<0.001$). The neuromuscular junction data supports the muscle atrophy data, in that the autograft group exhibited greater functional recovery at 180 days.

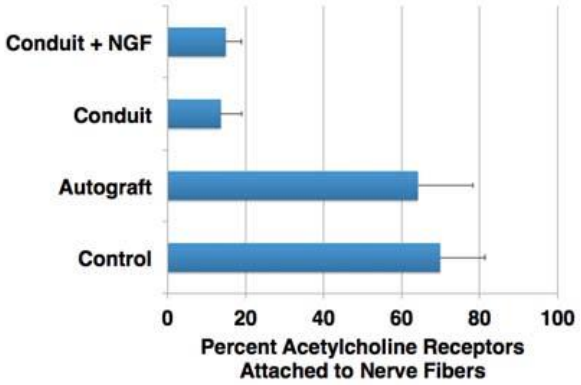


Figure 13. *The autograft and control group had more acetylcholine receptors attached to nerve fibers than the conduit and conduit and NGF groups.*

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

1. Continuation of PhD research project for Pratima Labroo.
2. Continuation of Surgical Resident research project for Kyle Edwards
3. Continuation of MS research project for Scott Ho.
4. Undergraduate research project for Megan Roach, Rainey Cornaby, and Artemis Sefandonakis

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to Report

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Nothing to Report

4. IMPACT: Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

One of the key impacts of the present approach is that it is possible to easily manipulate our nerve conduit device design. That is through the use of our mathematical model it is possible to predict what size reservoir and what size hole is required to release a set amount of a drug or growth factor. This was demonstrated by our ability to quickly redesign our device from PLGA to PTFE (data not reported). Without much troubleshooting we were able to release fluorescently labeled dextran from PTFE, in a manner that was consistent with our model.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

As part of this work, we have developed a new mathematical model that can be used by researchers to predict reservoir volume, drug amount, drug concentration, and diffusion hole size. This model will help researchers to avoid costly and time intensive in-vitro trials. We have developed fabrication and sterilization protocols for a nerve conduit device with a drug reservoir and tested the efficacy of the device using in-vitro and DRG studies. This data will help researchers/industry to further develop drug delivery efforts in other areas as well.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*

- *adoption of new practices*

Nothing to Report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Nothing to Report

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Nothing to Report

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. PRODUCTS: List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  1. Keng-Min Lin, Jill E. Shea, Bruce K. Gale, Himanshu Sant, Patti Larrabee and Jayant Agarwal. "Nerve growth factor release from a novel PLGA nerve conduit can improve axon growth", submitted to Journal of Micromechanics and Microengineering, acknowledgement of federal support (yes)
  2. Keng-Min Lin, Jill E. Shea, Bruce K. Gale, Himanshu Sant, Srinivas Chennamaneni, Michael Burr and Jay Agarwal. PDMS drug delivery devices: potential application in nerve regeneration, Biomedical Microdevices, in preparation, acknowledgement of federal support (yes)

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Keng-Min Lin, IMPLANTABLE DEVICES FOR SENSING AND DRUG DELIVERY IN OPHTHALMOLOGY AND RECONSTRUCTIVE SURGERY, Ph. D. Dissertation, Department of Mechanical Engineering, University of Utah, May 2014, acknowledgement of federal support (yes)

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Scott Ho, Pratima Labroo, Keng-Min Lin, Himanshu Sant, Jill Shea, Jay Agarwal, Bruce Gale, Bioresorbable Multi-Drug Delivery Conduit to Promote Peripheral Nerve Regeneration, in Proceedings of 2014 BMES Annual Meeting, San Antonio, Texas, October 22-25, 2014.

Pratima Labroo, Jill E Shea, Himanshu Sant, Bruce Gale, and Jayant Agarwal, Controlled Delivery of Growth Factors and Small Molecules for Peripheral Nerve Regeneration, oral presentation at 2015 AIChE Annual Meeting November 10, 2015.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  http://www.mems.utah.edu/publications/
  This website lists the publications and research originating from Co-PI Dr. Gale's lab.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Fabrication of biodegradable drug delivery prototypes using PLGA. We will publish journal articles to share the device fabrication techniques.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  No new inventions as a result of this grant. We had a patent filed prior to receiving this grant.

SANT HIMANSHU JAYANT, GALE BRUCE KENT, AGARWAL JAYANT P, LIN KENG-MIN, METHODS AND DEVICES FOR CONNECTING NERVES, Last status change: 2013-05-10/ Fill date:2012-10-16, WO 2013066619

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*

- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

1. Mathematical model based on Fick's diffusion law
2. Fabrication of dual chamber combined PLGA nerve guide and drug delivery device prototypes
3. Use of laser to create diffusion hole

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: *5*

Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support: *The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Personnel	**Role**	**Percent Effort**	**Months**
Jay Agarwal	PD/PI	7.2	0.6
Overall management of the project, guidance to students, weekly meetings and report preparation.			
Bruce Gale	Co-I	7.2	0.6
Device manufacturing, weekly meetings			
Himanshu Sant	Co-I	14.4	1.2
Device manufacturing and validation, mathematical model, weekly meetings and report preparation.			
Bala Ambati	Co-I	7.2	0.6
ELISA, troublshooting			
Jill Shea	Collaborator	14.4	1.2
IACUC/ACURO approvals, ELISA, histology, animal studies, weekly meetings and report preparation.			
Pratima Labroo	Student	28.8	2.4
Device manufacturing, histology, ELISA, weekly meetings			

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

No Change

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. **APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Drug Delivery for Peripheral Nerve Regeneration

PR121391
W81XWH-13-1-0337

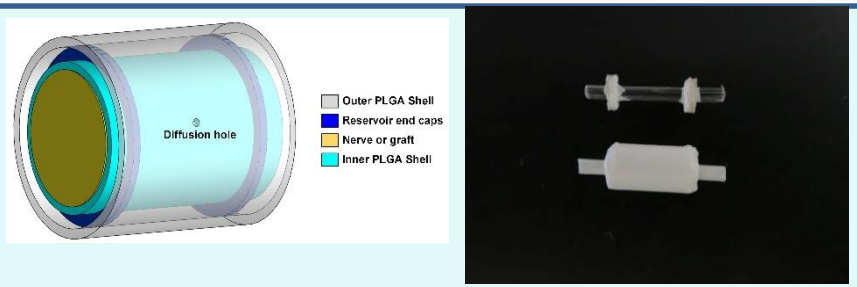
PI: Jayant Agarwal **Org:** University of Utah **Award Amount: Direct** $125,000; **Total** $186,250

Study Aims

- To optimize release kinetics of nerve growth factor (NGF) in vitro using our novel drug delivery conduit.
- To evaluate the effectiveness of the conduit-drug delivery device to enhance nerve regeneration across a 15mm nerve gap in a rat sciatic nerve model.

Approach

This proposal focuses on a novel method for drug delivery to the regenerating axons. We will study the efficacy of novel biodegradable nerve conduits in (1) continuously delivering small molecules or growth factors to the regenerating axons at a controlled rate and (2) improving the degree of axon regeneration and functional recovery. These nerve conduits are unique in that the desired drug is released from an integrated biodegradable reservoir through diffusion hole into the conduit lumen. This efficacy study will utilize a Sprague Dawley rat sciatic nerve gap model: 15 mm gap.



(Left) Schematic of nerve drug delivery: Inner (light blue) and outer (grey) PLGA shell, drug reservoir (space between shells), and end caps defining boundary of reservoir (dark blue.) The diffusion hole (in the inner shell) can be modified to control the rate of diffusion. (Right Top) Inner conduit with two end caps defining the outer boundary of the reservoir. (Right Bottom) Completed PLGA device with inner and outer tube, as well as a diffusion hole on the inner conduit that enables the release of NGF from the reservoir into the inner chamber.

Accomplishment: We have manufactured the devices, implanted in an animal model, and harvested all of the experimental groups.

Timeline and Cost

Activities CY	Sep'13-Aug'14			Sep'14 - Mar'15
Aim 1.1. Manufacturing of the drug delivery device-nerve guide conduit combination for 15 mm gap including optimization of PLGA ratios and reservoir dimensions				
Aim 1.2 – Optimization of nanoporous membrane				
Aim 1.3 – Confirmation of near-zero-order diffusion kinetics in vitro in a diffusion chamber of NGF and ELISA detection				
Aim 2.1 - IACUC approval, obtain N=48 animals				
Aim 2.2 - Implant devices in animal groups 1-3, sacrifice of half animals at 21 days, NGF quantification				
Aim 2.3 -Walking track, sacrifice of half animals at day 90, explanted tissue analysis, NGF quantification				
Estimated Budget ($)	**$63K**		**$63K**	**$60K**

Goals/Milestones (Example)

CY Sep'13-Aug'14 Goal – Optimize release kinetics of NGF *in vitro*

☑Prototype fabrication
☑Filter optimization: porosity, pore size and dimensions
☑Reservoir optimization: conduit dimensions
☑Optimization of NGF

CY Sep'14-Mar'15 Goal – Determine device efficacy with Sprague Dawley rat animal sciatic nerve gap model

☑Implant drug delivery device and compare with an autograft and empty nerve conduit
☑ Walking track, muscle atrophy, histology

Comments/Challenges/Issues/Concerns

- None

Budget Expenditure to Date

Projected Expenditure: $125,000
Actual Expenditure: $186,250 ($125,00 Direct + $61,250 Indirect) as of October 31, 2015

Updated: November 28, 2015